

Minimal homology requirements for PCR primers

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Homologous genes from different species may be successfully cloned by utilizing the PCR process (1-3). Since this becomes an increasingly attractive alternative to conventional low stringency screens, it is interesting to know which minimal homology a prime needs in order to be effective for the PCR process. We have addressed this question by testing a preexisting set of primers on defined DNA templates. The latter were obtained from subcloned pieces of the *hunchback* genes of *Drosophila melanogaster* and *Drosophila virilis* (4). In the tests we used always one primer with a perfect homology and one primer with a partial homology in order to amplify pieces with a length of 200-1000nt. The amplification products were resolved on agarose gels. Table 1 summarizes the results. Primers with a length between 17-20nt need at least three homologous nucleotides at their 5'-end for successful priming. Weak priming was however also obtained with a long primer having only 2 nucleotides homology (Table 1,n). Surprisingly, the remainder of the primer needs only very little homology with the target sequence. This is particularly clear from the cases c,d and k (Table 1).

From these results we suggest the following criteria for primers to be employed in cloning homologous genes: the length should be preferably between 20-24nt and the three 3' nucleotides should match completely. This may be ensured by letting them end at a Met. or Trp residue or by synthesizing appropriate redundant oligos for these positions. For the rest of the primer it appears to be sufficient to conform largely with the preferred codon usage of the organism. This allows to reduce the redundancy of the employed primers which should in turn reduce the amount of artefacts. Using primers based on these criteria we have successfully cloned the *hunchback* gene from *Musca domestica* (unpublished).

	TCGCACATCGCAGCTA	
a	---C---T---G	-
b	CCTCCGACCCAGGCT	-
	---GA-----A	-
c	GTGG--AT--A-C-A---	+
d	ATCA--AT-TG-T---	+
e	ATCTATATACGAGC	-
f	G-----C-A-A---	-
g	TCCCAAGAACGCCAGT	-
h	---A-G-C---C---	-
i	CTGGCCGCGACGCTTGG	-
j	GC-----T-G---	++
k	AG--TGT-----	++
l	TGCT--TC-C-----	+
m	G-CC-TT--GT-C---	+
n	AAGTATGTCACAGCC	++
o	---A-----C---	++
p	TTGTGGAGCCAGATGTGGC	++
q	A-----GT--G-G-C---	-
r	GTCGAGATGATGACCTGCTCTCTGTTGATATTGC	+
s	A-GAT---CGACGG-----C-----G-G---	+

Table 1: Primers employed in this study. The homologous regions are indicated in italics. Dashes represent identities. "+" means good, "++" weak and "-" no amplification. The PCR was set up as described (1), using cycles of 1 min at 95°, 2 min at 37° and 1 min at 72° for 25 cycles. 0.1ng of linearized plasmid DNA containing the respective gene regions were used as templates. Primers f and m were retested in cycles with annealing at 30° and elongation at 65°, but remained negative.

References: (1) Saiki, R.K. et al. (1988) *Science* 239, 487-491. (2) Gould, S.J., Subramani, S. & Scheffler, I.E. (1989) *PNAS* 86, 1934-1938. (3) Libert, F. et al. (1989) *Science* 244, 569-572. (4) Treier, M., Pfeifle, C. & Tautz, D. (1989) *EMBO J.* 8, 1517-1525.

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g	TCGACGAGCAGCAGT	
h	---A---G---C---C	-
i	CTGGCCGCGAGCTTAG	
j	GC---A---T---G	++
k	AG---TCG---C	++
l	TCCT---TC---C	+
m	G-C---TT---GT---C	+
n	AGTATGTCACAGCC	
o	---A---C---C	++
p	TTGTGGAGCGCATGTGGC	
q	A---GT---G---C---C	++
r	GTCGAGATGATGACCTGGCTCCTGTTTATATTTC	
s	A-GAT---CGACAGG---C---G---C	+

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